IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application No. 10/501,628

Confirmation No. 2223

Applicant: Martin et al.

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Examiner: Michael D. Burkhart

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132 OF MATTHEW D. SCHARFF, M.D.

I, Matthew D. Scharff, do hereby declare:

1. I am a co-inventor of the above-referenced patent application, and a Professor in the Department of Cell Biology at the Albert Einstein College of Medicine of Yeshiva University. I am a member of the Scientific Advisory Board of AnaptysBio, Inc., which is a licensee of the present application, as well as a stockholder in AnaptysBio, Inc. I have over 20 years of experience in molecular immunology and am an expert in this field as illustrated in by my *curriculum vitae,* which is attached hereto as Exhibit A.

2. As early as 2001, somatic hypermutation (SHM) was known as one of the primary mechanisms by which antibody diversification occurs *in vivo*. SHM involves a programmed process of mutation of variable regions of rearranged immunoglobulin genes that creates additional diversity within an expanding clone of B cells responding to an antigen. Specifically, following antigen recognition by B cells, a B cell enters the germinal center of peripheral lymphoid organs to become a centroblast B cell. In the germinal center, SHM occurs at rates of 10^{-5} to 10^{-3} mutations per base pair per generation, which is ~ 1 million-fold higher than the spontaneous rate of mutation in most other genes. Generally, the mutations are single base substitutions, with occasional insertions and deletions. While mutations occur throughout the rearranged V regions, the mutations are preferentially targeted to "hot spots" having the sequence WRCY (W=A or T, R=A or G, and Y=T or C) or

WA motifs. Transition mutations arise more frequently than transversion mutations (see, e.g., Diaz et al., *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 356(1405): 67-72 (2001)).

3. As early as 2001, class switch recombination ("CSR") (also known as isotype switching) was known to be a mechanism by which the isotype (or class) of an antibody is changed (e.g., from IgM to IgG). During CSR, a portion of the antibody heavy chain locus is removed from the chromosome, and the gene segments surrounding the deleted portion are rejoined to retain a functional antibody gene that produces an antibody of a different isotype. Double-stranded breaks are generated in DNA at conserved nucleotide motifs, called switch (S) regions, which are upstream from gene segments that encode the constant regions of antibody heavy chains; these occur adjacent to all heavy chain constant region genes with the exception of the δ-chain (see, e.g., Janeway et al. (eds.), *Immunobiology*, 5th ed., Garland Publishing, New York, NY (2001)).

4. Although by 2001 it was well known that SHM and class switch recombination occur in B cells at the same stage of differentiation, it was also known as early as 2001 that SHM and CSR appeared to be very different and distinct biochemical processes. The hypothesis that SHM and CSR are independent processes was supported by evidence suggesting that different molecules were involved in SHM and CSR. For example, as early as 2001, CSR was found to require the enzyme DNA-PK$_{CS}$, which is dispensable for SHM (see, e.g., Bemark et al., *J. Exp. Med.*, *192*(10): 1509-1514 (2000)). However, several molecules were postulated to be involved in the regulation of both SHM and CSR, including activation-induced cytidine deaminase (AID) (see, e.g., Murumatsu et al., *J. Biol. Chem.*, *274*(26): 18470-18476 (1999), and Murumatsu et al., *Cell*, *102*: 553-563 (2000)), mismatch repair (MMR) proteins (see, e.g., Poltoratsky et al, *J. Exp. Med.*, *192*: F27-F30 (2000)), and error-prone DNA polymerases (see, e.g., Poltoratsky et al., *supra*).

5. As a result, by 2001 it was hypothesized that SHM and class switch recombination were the result of defects in DNA polymerases involved in DNA damage repair, rather than an increased mutation rate (see, e.g., Zeng et al., *Nat. Immunol.*, *2*: 537-541 (2001), Rogozin et al., *Nat. Immunol.*, *2*: 530-536 (2001), and Zan et al., *Immunity*, *14*: 643-653 (2001)).

6. At this time, AID was known to be a homolog of the APOBEC1 protein, which is a member of the RNA editing cytidine deaminase protein family (Muramatsu et al., *J. Biol. Chem.*, *274*(26): 18470-18476 (1999)) that is expressed in centroblast B cells.

7. Murumatsu et al., *Cell*, *102*: 553-563 (2000) ("the Murumatsu reference"), which is cited in the Office Action, discloses that overexpression of AID in a lymphoma cell line augments antibody class switching from IgM to IgA without cytokine stimulation. The Muramatsu reference also discloses the generation of AID-deficient mice. AID deficiency completely blocked CSR in B cells activated by lipopolysaccharide (LPS) *in vitro* and by antigens *in vivo*. In addition, the Muramatsu reference demonstrates that B cells isolated from AID-deficient mice are defective in somatic hypermutation. Based on these results, the Muramatsu reference hypothesizes that AID is an RNA editing enzyme that requires a co-factor for its activity (Muramatsu reference at page 560, first column, and page 561, first column).

8. The conclusions drawn by the Murumatsu reference with respect to the potential role of AID in SHM are based solely on the phenotype of AID knock-out mice. While the Murumatsu reference describes experiments in which AID is overexpressed in mouse B cells, it reports only on the effects of AID overexpression on CSR and not SHM.

9. As of 2001, there were numerous examples of situations in which the phenotype of a gene knock-out (e.g., in a mouse) was difficult to interpret, and even contradicts the phenotype observed when the same gene is overexpressed in a cell or organism. For example, a deficiency in the 5-hydroxytryptamine (5-HT) 1B gene leads to hyperaggressive behavior in mice (see, e.g., Gingrich et al., *Current Opinion in Neurobiology*, *10*: 146-152 (2000)); however, 5-HT antagonists have no affect on aggressive behavior. The Gingrich reference also states that the phenotypes of knockout mice are difficult to interpret due to a variety of factors, such as development and compensatory changes, the influence of the gene knockout on nearby genes, the effect of the genetic background strain, maternal behavioral influences, and pleiotropy. In another example, mice with a complete deficiency in insulin-like growth factor-1 (IGF-1) exhibit postnatal lethality, growth retardation, infertility, and defects in the development of major organ systems (see, e.g., Liu et al., *P.S.E.B.M.*, *223*: 344-351 (2000)). In contrast, conditional deletion of IGF-1

in mouse liver tissue using the Cre-Lox system produces no defects in growth or development (Liu et al., *supra*).

10. Thus, as of 2001, AID was one of many factors suspected to be involved in SHM and class switch recombination, but was not reported as the key enzyme responsible for induction of SHM. Moreover, at this time, the precise mechanism of action of activation-induced cytidine deaminase (AID) was unknown, inasmuch as there was evidence suggesting that AID was an RNA editing enzyme (see, e.g., the Murumatsu reference) and that AID acted directly on DNA (see, e.g., Jacobs et al., *Curr. Opin. Immunol.*, *13*(2): 208-218 (2001)). Indeed, Poltoratsky et al., *supra*, states that the mechanism of action of AID was unclear in 2000, and V region mutation still occurs in mice and humans with defects in AID.

11. In 2001, it was not known nor suggested that AID alone is sufficient to induce SHM. In fact, such an idea would have been greeted with significant skepticism because of the complexity of SHM and class switch recombination processes, the unique restriction of these processes to the Ig locus, and the many other factors thought necessary to enable somatic hypermutation and class switch recombination *in vivo*, as described above. Indeed, one of ordinary skill in the art would not have suspected that AID alone is sufficient to induce SHM.

12. In view of the foregoing, as of 2001 there was no reason to believe that AID actually induces mutations in DNA that lead to SHM and class switch recombination. Moreover, there was no reason to believe that AID is capable of selectively targeting mutation of DNA sequences within the Ig locus. In addition, one could not accurately predict the function of a particular protein based solely on the phenotype produced when the gene encoding the protein is disrupted or deleted.

13. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 10/19/09

Exhibit A

Principal Investigator/Program Director (Last, First, Middle): Scharff, Matthew D.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2. Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
MATTHEW D. SCHARFF	Professor of Cell Biology
eRA COMMONS USER NAME Matthew	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Brown University	B.A.	1954	Biology
NYU College of Medicine	M.D.	1959	Medicine

Positions Held: House Officer, 2nd and 4th (Harvard) Medical Services, Boston City Hospitals, Boston, MA (1959-1961); Research Associate NIAID, Bethesda, MD (1961-1963); Associate (1963-1964); Assistant Professor (1964-1967), Associate Professor (1967-1971), and Professor (1971-), Departments of Cell Biology and Medicine; Chairman, Department of Cell Biology (1972-1983); Director, Division of Biological Sciences (1975-1981); Associate Director (1975-1986), Director (1986-1995) and Deputy Director (1995-2003) of the Cancer Center; and Harry Eagle Professor of Cancer Research (1983-), Distinguished Professor (2006-) all at the Albert Einstein College of Medicine.

Past Outside Activities: Study Section & Panels for the NIH, NSF, ACS, American Heart Association Study Section; Council of the American Cancer Society; Scientific Advisory Committee, Scalvo Research Institute, Siena, Italy; Scientific Advisory Board, Genetic Systems; President, Harvey Society; Chairman of the Board of Scientific Counselors for the Division of Cancer Biology and Diagnosis, NCI; Board of Trustees, Cold Spring Harbor Laboratory; Outside Advisory Committee for Guthrie Foundation Laboratory. Outside Advisory Committees for Cancer Centers at Fox Chase, Duke, U. of Colo. and Columbia U, Univ. of Pennsylvania and and MD Anderson. Scientific Advisory Boards of the Jane Coffin Childs Foundation, The Irvington Institute and the Howard Hughes Medical Institute. Advisory Committee to the Director of the NIH; Bioscience Advisory Committee, Johnson and Johnson; Scientific Advisory Committee, N.Y. Blood Center, the Ludwig Institute for Cancer Research and the Aaron Diamond AIDS Research Center of the City of New York Cancer Center; Research Program Advisory Committee of the Multiple Sclerosis Society; Co-Chairman, Board of Scientific Counselors, Division of Basic Sciences, NCI; member of NCI Executive Committee; Advisory Committee to the Director of the NCI, 1996-2000, Outside Advisory Committee for the Cancer Center the The Simons Arthritis Center, U. of Texas, Southwestern Medical School, General Motors Sloan Award Selection Committee, Vice Chair 2000, Chair 2001;. Chairman, Scientific Advisory Board, Rappaport Family Institute, Haifa, Israel; Member, National Research Council Committee on Research Standards and Practices; Member, General Motors Awards Assembly; OMRF Scientific Board of Visitors.

Present Outside Activities: Outside Advisory Committees for the City of Hope, NIEHS Center of Environmental Medicine and the Cancer Center at N.Y.U. and the; Scientific Advisory Board, Helen Hay Whitney Foundation;

Honors: Phi Beta Kappa; Sigma Xi; AOA; American Assoc. of Immunologists; American Society for Clinical Investigation; Harvey Lecture 1974: Solomon A. Berson Alumni Achievement Award for Basic Sci. from N.Y.U. 1980; Dyer Lecture; NIH 1981; National Academy of Sciences 1982; The Lymphoma Foundation Award 1982; American Academy of Arts and Sciences, 1984; Outstanding Investigator Award, NCI, (1985 & 1992); N.Y. Academy of Medicine Award for Distinguished Contributions to Biomedical Sceince 1990; Albert Einstein College of Medicine Commemorative Award (1993); Stewart Lecture, Uniformed Services University of the Health Sciences, 1993, Doctor of Medical Science (Hon) Brown U., 1994, Duke University Award for Excellence in Immunologic Research, 1994. American Association of Immunologists Award for Excellence in Mentoring, 1998; Lifetime Achievement Award for Outstanding Teaching, Albert Einstein College of Medicine, 2002; Honorary Alumnus, AECOM, 2002; Donald A. Rowley Award for Outstanding Mentoring, U. of Chicago, 2003; Mayor of NY's Lifetime Achievement Award for Excellence in Science and Technology, 2003; the Albert Einstein College of Medicine Marshall S, Horwitz Faculty Prize for Research Excellence, 2006. Albert Einstein Faculty Mentoring Award 2007.

Selected Recent Publications (from 270)

1. The G-U mismatch glycolylase Mbd4 is dispensable for somatic mutation and class switch recombination. Philip D. Bardwell, Alberto Martin, Edmund Wong, Ziqiang Li, Winfried Edelmann and **Matthew D. Scharff.** J. Immunol. 1701620-1624, 2003
2. Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of RNase. Ronda Bransteitter, Phuong Pham, **Matthew D. Scharff** and Myron F. Goodman. Proc. Natl. Acad. Sci. USA 100: 4102-4107, 2003
3. Induction of somatic hypermutation is associated with modifications in immunoglobulin variable region chromatin. Caroline J. Woo, Alberto Martin, and **Matthew D. Scharff** Immunity 19:479-489, 2003
4. Msh2 ATPase Activity is Essential for Somatic Hypermutation at A-T Basepairs and for Efficient Class SwitchRecombination Alberto Martin, Ziqiang Li, Diana Lin, Philip D. Bardwell, Maria D. Iglesias-Ussel Winfried Edelmann, and **Matthew D. Scharff**. J.Exp.Med. 198:1171-1178, 2003
5. Altered somatic hypermutation and reduced class switch recombination in Exonuclease 1-mutant mice. Philip D Bardwell, Caroline J Woo, Kaichun Wei, Ziqiang Li, Alberto Martin, Stephen Z Sack, Tchaiko Parris, Winfried Edelmann, and **Matthew D Scharff** Nat. Immunol. 5: 224-229, 2004
6. The generation of antibody diversity through somatic hypermutation and class switch recombination. Li, Z.Woo, C. J. Iglesias-Ussel, M.D., Ronai, D., **Scharff, M. D.** Genes and Development, 18:1-11, 2004
7. Examination of Msh6- and Msh3-deficient Mice in Class Switching Reveals Overlapping and Distinct Roles of MutS Homologues in Antibody Diversification. Li Z, Scherer SJ, Ronai D, Iglesias-Ussel MD, Peled JU, Bardwell PD, Zhuang M, Lee K, Martin A, Edelmann W, **Scharff MD**. *J Exp Med.* **200**, 47-59, 2004
8. Differential regulation of histone acetylation and generation of mutations in switch regions is associated with Ig class switching Li, Z, Luo, Z and **Scharff, M.D.** Proc Natl Acad Sci U S A.101:15428-33. 2004
9. The role of activation-induced cytidine deaminase in antibody diversification, immunodeficiency, and B-cell malignancies. Luo, Z, Ronai, D, and **Scharff, M.D.** J Allergy Clin Immunol. 114:726-35; 2004
10. Methylation protects cytidines from AID-mediated deamination. Larijani, M., Frieder, D., Sonbuchner, T. M., Bransteitter, R., Goodman, M. F., Bouhassira, E. E., **Scharff, M. D.**, Martin, A. Mol Immunol. 42:559-60 2005
11. Identifying protein-protein interactions in somatic hypermutation. Goodman, M. F., **Scharff, M. D.** J Exp Med. 493-6, 2005
12. *Cis*-acting sequences in the IgH gene can regulate somatic mutation in hybridoma cells. D. Ronai, M.D. Iglesias-Ussel, M. Fan, M.J. Shulman, **M.D. Scharff**. PNAS. 102:11829-11834, 2005
13. A role for Mlh3 in somatic hypermutation. Ziqiang Li , Jonathan Peled, Chunfang Zhao, Anton Svetlanov, Diana Ronai, Paula E. Cohen, and **Matthew D. Scharff**. DNA Repair. 5:675-682, 2006
14. The mismatch repair protein Msh6 plays a role in the targeting of AID to the Ig locus *in vivo*. Z. Li, C. Zhao, Z. Polonskaya, M. Zhuang, G. Yang, M.D. Iglesias-Ussel, Z. Luo, W. Edelmann, and **M.D. Scharff**. Immunity 24:393-403, 2006
15. Expression of AID facilitates the generation of class switch variants from hybridoma cells. M.D. Iglesias-Ussel, A. Martin, M. Fan, Z. Li, **M.D. Scharff**. J. Immunol. Meth. 316:59-66, 2006
16. Mouse models revealed the mechanisms for somatic hypermutation and class switch recombination of immunoglobulin genes. Maria D. Iglesias-Ussel, Ziqiang Li, and **Matthew D. Scharff**. In "The Mouse in Biomedical Research", 2nd edition. J. G. Fox et al eds. Vol 4, pages 155-168. 2007.
17. Targeting AID to the Ig genes. Mechanisms of Lymphocyte Activation and Immune Regulation. Li Z, Luo Z, Ronai D, Kuang FL, Peled JU, Iglesias-Ussel MD, **Scharff M.D.** Adv Exp. Med. Biol. 596:93-109, 2007
18. Detection of Chromatin-Associated ssDNA in Regions Targeted for Somatic Hypermutation. Ronai, D., Iglesias-Ussel. M.D., Fan, M., Li, Z., Martin, A and **M. D. Scharff.** J. Exp. Med. 204:181-190, 2007
19. AID initiated purposeful mutations in immunoglobulin genes. Myron F. Goodman, **Matthew D. Scharff** and Floyd E. Romesberg. Advances in Immunology. 94:127-155, 2007
20. Human recombinant Fab fragments with sub-nanomolar affinities for acetylated histones. Batova, I, C. Kowal, R. May, **M. D. Scharff,** and B. Diamond. J. Immunological Methods. 329:1-10. 2008
21. The Biochemistry of Somatic Hypermutation. Peled, J.U., F. L. M. D. Kuang, Iglesias-Ussel, S. Roa, S.L. Kalis, M.F. Goodman and M.D. Scharff. Annual Reviews of Immunology. 26:481-511, 2008
22. Does antisense make sense of AID targeting? Roa, S, Kuang, FL, and **Scharff, M.D.**, Proc. Natl. Acad. Sci. USA 105: 3661-2, 2008

23. Ubiquitylated PCNA plays a role in somatic hypermutation and class-switch recombination and is required for meiotic progression.Roa S, Avdievich E, Peled JU, Maccarthy T, Werling U, Kuang FL, Kan R, Zhao C, Bergman A, Cohen PE, Edelmann W, Scharff MD. Proc Natl Acad Sci U S A. 105:16248-53. 2008
24. SHMTool: A webserver for comparative analysis of somatic hypermutation datasets. Maccarthy T, Roa S, Scharff MD, Bergman A. DNA Repair (Amst). 8:137-41, 2009

OTHER SUPPORT

SCHARFF, Matthew D.

ACTIVE

1RO1CA72649-10 (M. D. Scharff) 09/15/97 - 2/28/09 3.0 Calendar
NIH $237,113

Somatic Mutation of Ig Variable Region

The major goal is to use transfected and manipulated Ig genes to study the role of chromatin acetylation and cis-acting sequences in V region hypermutation.
Role: PI

2R01 CA102705 (M.D. Scharff) 01/6/2009 – 1/7/2013 1.8 Calendar
NIH $170,886 (direct)

Role of Mismatch Repair in V region mutation and isotype switching

This study provides new insights into the biochemical mechanisms of SHM and CSR and the role of MMR on predisposing to B cell malignancies and other cancers.
There is no overlap in the grant and the current application. On MMR all in mice
Role: PI
Extended without additional funding and pending

U54 AI57158 (M.D. Scharff) 09/04/03 - 02/28/09 0.6 Calendar
NIH Health Research Inc. (2162-03) $197,064
Hybridoma Facility/NBC Monoclonal Antibody Core
Dr. Scharff will receive 5% of his salary as the Director of the Hybridoma Facility.
Role: PI

2 P30 CA13330-35 (Goldman, I.D.) 06/01/77 - 06/30/08 0.6 Calendar
NIH $7,969
Core Support for Cancer Research Media preparation
Role: Leader of the Program in Immunooncology (5%)

Pending

1 R01 CA102705A1 (M.D. Scharff) 07/01/08 - 4/30/13 1.8 Calendar
NIH $189,873
Resubmission received a 11.8% and awaits funding decision

U54 AI57158 (M.D. Scharff) 09/04/03 - 02/28/09 0.6 Calendar
NIH Health Research Inc. (2162-03) $160,000
Co-PI on 4 projects to make better monoclonal antibodies